VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph beginning at line 28 of page 24 has been amended as follows:

Expression control sequences that are suitable for use in a particular host cell are often obtained by cloning a gene that is expressed in that cell. Commonly used prokaryotic control sequences, which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta-lactamase (penicillinase) and lactose (*lac*) promoter systems (Change *et al.*, *Nature* (1977) 198: 1056), the tryptophan (*trp*) promoter system (Goeddel *et al.*, *Nucleic Acids Res.* (1980) 8: 4057), the *tac* promoter (DeBoer, *et al., Proc. Natl. Acad. Sci. U.S.A.* (1983) 80:21-25); and the lambda-derived P_L promoter and N-gene ribosome binding site (Shimatake *et al.*, *Nature* (1981) 292: 128). The particular promoter system is not critical to the invention, any available promoter that functions in prokaryotes can be used. Standard bacterial expression vectors include plasmids such as pBR322-based plasmids, *e.g.*, pBLUESCRIPT™, pSKF, pET23D, λ-phage derived vectors, and fusion expression systems such as GST and LacZ. Epitope tags can also be added to recombinant proteins to provide convenient methods of isolation, *e.g.*, c-myc, HA-tag, 6-His (SEQ ID NO:30) tag, maltose binding protein, VSV-G tag, anti-DYKDDDDK (SEQ ID NO:31) tag, or any such tag, a large number of which are well known to those of skill in the art.

Paragraph beginning at line 22 of page 31 has been amended as follows:

Based on the published amino acid sequence of Sso7d, seven oligonucleotides were used in constructing a synthetic gene encoding Sso7d. The

oligonucleotides were annealed and ligated using T4 DNA ligase. The final ligated product was used as the template in a PCR reaction using two terminal oligonucleotides as primers to amplify the full-length gene. By design, the resulting PCR fragment contains a unique EcoRI site at the 5' terminus, and a unique BstXI site at the 3' terminus. In addition to encoding the Sso7d protein, the above PCR fragment also encodes a peptide linker with the amino acid sequence of Gly-Gly-Val-Thr (SEQ ID NO:32) positioned at the C terminus of the Sso7d protein. The synthetic gene of Sso7d has the DNA sequence shown in SEQ ID NO:1, and it encodes a polypeptide with the amino acid sequence shown in SEQ ID NO:2.

Paragraph beginning at line 32 of page 31 has been amended as follows:

The synthetic gene encoding Sso7d was then used to generate a fusion protein in which Sso7d replaces the first 289 amino acid of Taq. The fragment encoding Sso7d was subcloned into a plasmid encoding Taq polymerase to generate the fusion protein, as follows. Briefly, the DNA fragment containing the synthetic Sso7d gene was digested with restriction endonucleases EcoRI and BstXI, and ligated into the corresponding sites of a plasmid encoding Taq. As the result, the region that encodes the first 289 amino acid of Taq is replaced by the synthetic gene of Sso7d. This plasmid (pYW1) allows the expression of a single polypeptide containing Sso7d fused to the N terminus of ΔTaq via a synthetic linker composed of Gly-Gly-Val-Thr (SEQ ID NO:32). The DNA sequence encoding the fusion protein (Sso7d-ΔTaq) and the amino acid sequence of the protein are shown in SEQ ID NOs:3 and 4, respectively.

Paragraph beginning at line 11 of page 32 has been amended as follows:

An Sso7d/full-length Taq fusion protein was also constructed. Briefly, a 1 kb PCR fragment encoding the first 336 amino acids of Taq polymerase was generated using two primers. The 5' primer introduces a SpeI site into the 5' terminus of the PCR

fragment, and the 3' primer hybridizes to nucleotides 1008-1026 of the Taq gene. The fragment was digested with SpeI and BstXI, releasing a 0.9 kb fragment encoding the first 289 amino acids of Taq polymerase. The 0.9 kb fragment was ligated into plasmid pYW1 at the SpeI (located in the region encoding the linker) and BstXI sites. The resulting plasmid (pYW2) allows the expression of a single polypeptide containing the Sso7d protein fused to the N terminus of the full length Taq DNA polymerase via a linker composed of Gly-Gly-Val-Thr (SEQ ID NO:32), the same as in Sso7d-ΔTaq. The DNA sequence encoding the Sso7d-Taq fusion protein and the amino acid sequence of the protein are shown in SEQ ID NOs:5 and 6 ~~SEQ ID. NO.5 and NO.6~~, respectively.

Paragraph beginning at line 29 of page 32 has been amended as follows:

Two primers were used to PCR amplify the synthetic Sso7d gene described above to introduce a Kpn I site and a NheI site flanking the Sso7d gene. The 5' primer also introduced six additional amino acids (Gly-Thr-Gly-Gly-Gly-Gly; SEQ ID NO:33), which serve as a linker, at the N terminus of the Sso7d protein. Upon digestion with KpnI and NheI, the PCR fragment was ligated into pPFKS at the corresponding sites. The resulting plasmid (pPFS) allows the expression of a single polypeptide containing Sso7d protein fused to the C terminus of the Pfu polymerase via a peptide linker (Gly-Thr-Gly-Gly-Gly-Gly; SEQ ID NO:33). The DNA sequence encoding the fusion protein (Pfu-Sso7d) and the amino acid sequence of the fusion protein are shown in SEQ ID NOs: 7 and 8, respectively.

Paragraph beginning at line 17 of page 33 has been amended as follows:

A fifth fusion protein joins a peptide composed of 14 lysines and 2 arginines to the N terminus of ΔTaq. To generate the polylysine (PL)-ΔTaq fusion protein, two 67 nt oligonucleotides were annealed to form a duplexed DNA fragment with a 5' protruding end compatible with an EcoRI site, and a 3' protruding end

compatible with an SpeI site. The DNA fragment encodes a lysine-rich peptide of the following composition: NSKKKKKKRKKRKKKGGGVT (SEQ ID NO:34). The numbers of lysines and arginines in this peptide are identical to the that in Sso7d. This DNA fragment was ligated into pYW1, predigested with EcoRI and SpeI, to replace the region encoding Sso7d. The resulting plasmid (pLST) expresses a single polypeptide containing the lysine-rich peptide fused to the N terminus of ΔTaq. The DNA sequence encoding the fusion protein (PL-ΔTaq) and the amino acid sequence of the protein are shown in SEQ ID NOs:11 and 12 ~~SEQ ID NOs: 11 and NO. 12~~, respectively.

Paragraph beginning at line 6 of page 39 has been amended as follows:

Lambda DNA (2.25 pM) was used as a PCR template. Three pairs of primers L71F (5'-CCTGCTCTGCCGCTTCACGC-3'; SEQ ID NO:13) and L71R (5'-GCACAGCGGCTGGCTGAG GA-3'; SEQ ID NO:14), L18015F (5'-TGACGGAGGATAACGCCAGCAG-3'; SEQ ID NO:15) and L23474R (5'-GAAAGACGA TGGGTCGCTAATACGC-3'; SEQ ID NO:16), and L18015F (5'-TGACGGAGGATAAC GCCAGCAG-3'; SEQ ID NO:17) and L29930R (5'-GGGGTTGGAGGTCAATGGGTTC-3'; SEQ ID NO:18), were used to amplify DNA fragments of the size of 0.9 kb, 5.5 kb and 11.9 kb, respectively. Each reaction contained 40 unit/ml of polymerase, where the unit was defined as described in Example 2, and 0.36 mM of each of the four dNTPs. The reaction buffer used for Pfu (from Stratagene) contained 20 mM Tris-HCl (pH 8.8), 2 mM $MgSO_4$, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, and 0.1 mg/ml BSA. The reaction buffer for Pfu-Sso7d, Taq, and Sso7d-Taq was the above buffer with an additional 40 mM of KCl. Two cycling programs with a 1 min or a 5 min extension time were used for PCR amplification. Each cycling program was composed of 94°C for 20 sec, hot start at 80°C by the addition of the polymerase, 20 cycles of 94°C for 10 sec followed by 72°C for 1 or 5 min, and 72°C for 5 min. The results showed that a Pfu-Sso7d fusion protein was able to amplify both the 1 kb and 5 kb fragments using a 1 min extension time, and was also able to amplify

the 10 kb fragment using a 5 min extension time. In contrast, Pfu polymerase amplified only the 1 kb fragment using either a 1 min or a 5 min extension time. Similarly, the Sso7d-Taq fusion protein amplified the 1kb fragment using a 1 min extension time, and both the 1 kb and 5 kb fragments with a 5 min extension time, whereas Taq polymerase amplified only the 1 kb fragment with a 5 min extension time.

Paragraph beginning at line 3 of page 40 has been amended as follows:

Lambda DNA (2.25 pM) was used as a PCR template. Four pairs of primers L71F (5'-CCTGCTCTGCCGCTTCACGC-3'; SEQ ID NO:13) and L71R (5'-GCACAGCGGCTGGCTGAG GA-3'; SEQ ID NO:14), L30350F (5'-CCTGCTCTGCCGCTTCACGC-3'; SEQ ID NO:19) and L35121R (5'-CACATGGTACAGCAAGCCTGGC-3'; SEQ ID NO:20), L2089F (5'-CCCGTATCTGCTGGGATACTGGC-3'; SEQ ID NO:21) and L7112R (5'-CAGCGGTGCTGACTGAATCATGG-3'; SEQ ID NO:22), and L30350F (5'-CCTGCCTGCCGCTTCACGC-3'; SEQ ID NO:23) and L40547R (5'-CCAATACCCGTTTCA TCGCGGC-3'; SEQ ID NO:24) were used to amplify DNA fragments of the size of 0.9 kb, 4.8 kb, 5.0 kb and 10.2 kb, respectively. Four concentrations (10 unit/ml, 20 unit/ml, 40 unit/ml and 80 unit/ml) of Pfu-Sso7d were used, and two concentrations (20 unit/ml and 40 unit/ml) of DyNAzyme EXT were used. Each reaction contained 0.36 mM of each of the four dNTPs. The reaction buffer for Pfu-Sso7d was as described in Example 6-1. The reaction buffer for DyNAzyme EXT contained 20 mM Tris (pH 9.0), 2 mM $MgCl_2$, 15 mM $(NH_4)_2SO_4$, and 0.1 % Triton X-100 (provided by Finnzymes). All reaction components were first mixed on ice, and the reactions were initiated by placing the sample plates into a thermal cycler (MJ Research) preheated to over 90°C. The PCR cycling program consists of 95°C for 20 sec, 20 cycles of 94°C for 10 sec and 70°C for 1 or 1.5 min, and 1 cycle of 72°C for 10 min.

Paragraph beginning at line 26 of page 41 has been amended as follows:

Human female or male type DNA (concentration, 1 fM) from placenta or chorionic tissue (from Sigma) was used as the template. Primers H-Amelo-Y (5'-CCACCTCATCCTGG GCACC-3'; SEQ ID NO:25) and H-Amelo-YR (5'-GCTTGAGGCCAACCATCA GAGC-3'; SEQ ID NO:26) were used to amplify a 212 bp amplicon from X chromosome and a 218 bp amplicon from Y chromosome. A single 212 bp fragment should be amplified from female typed DNA, whereas three fragments (212 bp, 218 bp, and the 212 bp/218 bp heterozygote) were expected from male typed DNA. Each reaction contained 20 unit/ml of polymerase and 0.36 mM of each of the four dNTPs. The reaction buffer for Taq included 10 mM Tris (pH 8.8), 50 mM KCl, 1.5 mM MgCl2 and 0.1% Triton X-100 (provided by Amersham). The reaction buffer for Pfu-Sso7d contains was performed using DyNAzyme EXT buffer (see Example 6-2) with an additional 40 mM KCl. All reaction components were mixed on ice, and the reaction was initiated by placing the plates into a thermal cycler preheated to above 65°C. The cycling program consisted of 95°C for 2 min, 30 cycles of 94°C for 5 sec, 64°C for 10 sec, and 72°C for 10 sec, followed by 1 cycle of 7 min at 72°C. Specific amplicons of expected sizes were amplified by both Pfu-Sso7d and Taq polymerase.

Paragraph beginning at line 12 of page 42 has been amended as follows:

Human DNA (1 fM) from placenta or chorionic tissue (from Sigma) was used as the template. Three pairs of primers, Bglbn536F (5'-GGTTGGCCAATCTA CTCCCAGG-3'; SEQ ID NO:27) and Bglbn536R (5'-GCTCACTCAGTGTGGCAAAG-3'; SEQ ID NO:28), Bglbn536F and Bglbn1083R, and Bglbn536F and Bglbn1408R (5'-GATTAGCAAAAGGGCCTAGCTTGG-3'; SEQ ID NO:29) were used to amplify DNA fragments of the size of 0.5, 1.1 and 1.4 kb, respectively. Each reaction contained 20 unit/ml of polymerase and 0.36 mM of each of the four dNTPs. The reaction buffers for Taq and Pfu-Sso7d were as described in Example 8-1. All reaction components were

first mixed on ice, and the reactions were initiated by placing the plates into a thermal cycler preheated to above 65°C. The cycling program consists of 95°C for 2 min, 30 cycles of 94°C for 45 sec, 64°C for 45 sec, and 72°C for 1 min, followed by 1 cycle of 7 min at 72C. With each of the three pair of primers used, an amplified product of the expected size was produced using Pfu-Sso7d. These results show that the specificity of amplification achieved by using Pfu-Sso7d is equal or better than that with Taq polymerase.